protein coat. The assembly of the COPI complex is initiated by the GTPase Arf1 in a nucleotide-dependent manner. After GDP/GTP exchange, soluble Arf1 is localized to the membrane by lipid flippase mediated by its myristoylated N-terminal amphipathic helix (myrAH) into the proximal leaflet of the Golgi membrane. The subsequent liberation of transport vesicles requires the full COPI complex and has been observed in vivo and in vitro. However, the role of Arf1 in the process of curvature induction has not been fully elucidated. To study the effects of Arf1 on membrane morphology we have evaluated binding and incorporation of recombinant S. cerevisiae Arf1p into lipid monolayers and bilayers. Using a Langmuir film balance setup and binding assays with artificial liposomes, we observe a myristoylation-dependent increase in membrane surface area upon addition of Arf1p. Confocal laser scanning microscopy and cryo electron microscopy reveal highly curved membrane structures upon incorporation of myristoylated Arf1p. Our results support a mechanism of positive curvature induction based on the bilayer couple theory.

1257-Pos Board B149
Ligand Binding Alters Dimerization and Sequestering of Urokinase Receptors in Raft-Mimicking Lipid Mixtures
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Urokinase-type plasminogen activator receptor (uPAR) is a glycosylphosphatidylinositol (GPI)-anchored protein that plays an important role in several physiological and pathological processes. Research on cellular systems has shown that certain uPAR-binding proteins, such as urokinase plasminogen activator (uPA) and vitronectin, may alter the functionally important uPAR dimerization, and sequestering of uPAR in the presence of lipid microdomains. Herein receptor sequestering information is obtained using XY-scans of confocal fluorescence intensity and dimerization data are acquired via photon counting histogram (PCH) analysis. According to our experiments, uPAR has a preference for cholesterol-enriched lipid domains and forms stable complexes of dimers in the presence of ligands. More importantly, the addition of ligands substantially influences the dimerization state and partitioning of the urokinase receptor in the membrane environment. The role lipid composition on receptor sequestering and dimerization will be discussed.

1258-Pos Board B150
The Role of SmXXXSm Motifs in Wild-Type and Ala391Glu FGFR3 Transmembrane Domain Dimerization
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Fibroblast growth factor receptor 3 (FGFR3) is a single pass membrane protein and a member of the receptor tyrosine kinase (RTK) family of proteins. FGFR3 is critically involved in cellular processes that regulate growth and development. FGFR3 has three distinct domains: the ligand binding extracellular domain, the cytosolic kinase domain, and the transmembrane domain (TMD). Previous work with the isolated FGFR3 transmembrane (TM) domain has shown that it has the ability to self-dimerize. Mutations in FGFR3 can lead to a variety of diseases, but more specifically, mutations in the TMD of the protein can cause achondroplasia (the most common form of dwarfism, thanatophoric (skeletal) dysplasia, Crouzon syndrome (cranial dysplasia) with acanthosis nigricans (hyperpigmentation of the skin), and bladder cancer. Though the structures of the extracellular and cytosolic domains of FGFR3 have been resolved, the structure of the TMD dimer and the effects of pathogenic mutations on the dimer structure are still unknown. Using the ToxR assay, structural studies were carried out by studying the role of the SmXXXSm motif in the dimerization of the FGFR3 TMD. The SmXXXSm motif has been shown to drive the dimerization of many transmembrane proteins. Results from the ToxR assay indicate that the SmXXXSm does not play a role in the dimerization of the WT FGFR3 TMD. In contrast, similar studies carried out with the A391E mutant TMD show that the SmXXXSm motif plays a role in TMD dimerization. These results lead to the conclusion that the pathogenic A391E TMD has a different dimer structure than the WT FGFR3 TMD.

1259-Pos Board B151
Plasmamembrane Organization in Signaling
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The composition of the plasma membrane has long been modeled as a fluid mosaic. (Singer and Nicolson, 1972) Studies in the last few years have identified micromodomains like lipid rafts and caveolae that constrain membrane proteins within a small region of the cellular plasma membrane. These domains facilitate anchoring of different signaling proteins, like H-Ras, that has been shown to co-localize with nano domains upon activation (Lommerse et al. 2005, Robl et al. 2004). It is believed that these nanodomains function as important platforms for a multitude of signaling cascades that are initiated at the plasma membrane. Given that many of the transmembrane signals will need a coordinated domain organization, it is of importance to investigate properties like size, shape, stability and their mutual interaction.

Here we transfected 3T3-cells to express the membrane anchor of H-Ras linked to Dendra2 or mEos2. Photo Activated Localization Microscopy (PALM) and Stimulated Emission Depletion Microscopy (STED) were used to make high resolution images of H-Ras distribution on the membrane as a probe for inner membrane domains. The GPI-anchored protein CD59 is used as a probe for the outer membrane domains to investigate colocalization of internal and external membrane domains. Using a SNAP-tag we covalently link Alexa647 to CD59 and image with direct stochastic optical microscopy (dSTORM).

1260-Pos Board B152
Polyunsaturated Free Fatty Acids Inhibit Coupling Between the Er Ca2+ Sensor STIM1 and the Ca2+ Channel Protein Orai1 Stimulated by IgE Receptor Crosslinking or Thapsigargin in a Process that Correlates with Disruption of Lipid Order
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Polyunsaturated fatty acids (PUFAs) have been found to be effective inhibitors of cell signaling in numerous contexts. We find that acute addition of these PUFAs in micromolar concentrations, including linoleic acid, substantially inhibits Ca2+ responses in mast cells stimulated by antigen-mediated crosslinking of FceRI or by the SERCA inhibitor, thapsigargin. In addition to inhibiting store-operated Ca2+ entry, linoleic acid inhibits antigen-stimulated release of Ca2+ from intracellular stores and granule exocytosis. Using AcGFP-Orai1 and STIM1-mRFP to monitor stimulated coupling in COS7 cells by FRET in a fluorometry assay, we find effective inhibition of this association by linoleic acid added either before or after stimulation by thapsigargin and ATP. Stimulation of bovine aorta cultured smooth muscle cells by norepinephrine, which does not inhibit FRET or Ca2+ signaling when added at the same concentrations. We showed previously that giant plasma membrane vesicles exhibit liquid order/liquid disorder phase separation at low temperatures, and we find that stearic acid enhances whereas linoleic acid prevents this phase separation. Our results suggest that PUFAs interfere with STIM1-Orai1 coupling by a mechanism that correlates with inhibition of membrane order heterogeneity in cells.

1261-Pos Board B153
Techniques for Direct Imaging of Nanoparticles in the Live Cell Plasma Membrane
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We recently developed a method termed TOCCSL ('Thinning out Clusters while Conserving Stoichiometry of Labeling') which allows for the first time the direct imaging of nanoscopic stable platforms with raft-like properties differing in the live cell plasma membrane. Our method senses these platforms by their property to assemble a characteristic set of fluorescent marker-proteins/lipids on a time-scale of seconds. A special photobleaching protocol was used to reduce the surface density of labeled mobile platforms down to the level of well-isolated diffraction-limited spots, without altering the single spot...
Stabilizing Lipid Domains
The Immunomodulator Enterotoxin Influences BCR Signaling by

Key Publication: Brameshuber et al., JBC 2010, 285(53): 411765-71

Adjuvants may potentiate immune response at the cell level by either enhancing receptor activation at the membrane or by increasing uptake of antigen. To investigate the mechanism behind the adjuvancy of LTIIb, a toxin that binds GM3, we studied its effects on the membrane structure, the mobility of BCR and B cell membrane signaling. biFC5S, a novel technique for probing interaction between membrane molecules and membrane domains, confirms that LTIIb pre-clusters cholesterol-stabilized domains. LTIIb, or its binding subunit alone, is found to reduce the mobility of BCRs, as measured by FRAP measurements and induce membrane signaling, as confirmed by calcium imaging. The calcium signaling pathway, however, seems to compete with that of BCR activation through IgM crosslinking. The B subunit of Choleratoxin (CTB), commonly used for labeling GM1, though also modulates cholesterol-stabilized domains, does not affect BCR mobility or trigger calcium signaling. These results show that GM1 and GM3, though both enriched in cholesterol-stabilized domains, behave differently, at least in CH27 B cells, upon crosslinking.

Quantifying the Effect of BCR Clustering on Plasma Membrane Organization
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The B cell antigen receptor (BCR) is an integral part of the adaptive immune system that communicates binding of antigen in the extracellular environment through the plasma membrane. Antibody binding to the BCR results in phosphorylation of intracellular tyrosine activating motifs (ITAMs), which subsequently bind to and activate numerous proteins involved in BCR regulation. Interestingly, many of the proteins regulating the early stages of the BCR signaling pathway are linked to the inner leaflet by saturated lipid anchors which tend to be associated with liquid-ordered membrane phase in model membranes. Also, the BCR becomes transiently detergent resistant following antigen-induced BCR clustering, suggesting that BCR clusters become coupled to membrane order following stimulation. In this work, we aim to characterize how BCR clustering could reorganize plasma membrane lipids by quantifying co-localization of BCR with fluorescent markers of liquid-ordered and liquid-disordered phases. We utilize two-color super-resolution fluorescence localization microscopy (STORM and PALM) in live and chemically fixed CH27 B cells to simultaneously image BCR and membrane anchored proteins, and we quantify their co-clustering using correlation functions. Our results from chemically fixed cells show that proteins anchored to the plasma membrane inner leaflet through saturated acyl-chain lipid modifications exhibit increased co-localization with BCR upon antigen stimulation, whereas those without lipid modifications or those anchored through branched acyl-chain modifications are not significantly co-localized with BCR before or after stimulation. These results are contributing to our long term goal of elucidating the role of lipid mediated interactions in the regulation of BCR signaling.

Eisosomes and Plasma Membrane Organization
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A vast body of evidence coming from different microscopy techniques has been instrumental in concluding the 10-year-long debate on whether biological membranes presented lateral segregation of proteins and lipids. Currently, the existence of membrane domains in both eukaryotes and prokaryotes has been common ground. However, the mechanisms that sustain membrane domain formation and maintenance remain largely unknown. Our work is focused on the study of Eisosomes, recently discovered plasma membrane domains in S. cerevisiae. In a first piece of work, we identified new eisosomal components and also showed that eisosomes are involved in sphingolipid metabolism (1). Thereafter, we showed that Pil1 and Lsp1, the major proteinaceous components of eisosomes, are able to form self-assemblies that bind and curvature membranes both in vivo and in vitro. We also showed that Lsp1 and Pil1 membrane-sculpting abilities are associated with the generation and organization of membrane domains (2). Thus, our currently published work support the hypothesis that a mechanism for membrane eisosome domain formation is membrane curvature generation directed by Pil1-Lsp1 assemblies.

B cell membrane is thought to contain transient spatial domains: cholesterol-stabilized lipid nano-domains and corals formed by interactions with the membrane cytoskeleton. Due to their small size and transient nature, these cannot be visualized directly and are challenging to characterize in intact cells. One possibility is to measure the diffusion of membrane proteins interacting with these domains. However, the diffusion should be measured with nanometer spatial and microsecond temporal resolution to correctly plot the protein’s path in the membrane. We show here that not only is the spatio-temporal resolution of thermal noise imaging (TNI) in an optical trap sufficient to plot the membrane protein’s path, but the trapping also allows gathering sufficient data within one small membrane area to “image” the membrane. We create for high resolutions maps of the local diffusion, local attraction potentials and membrane stiffness by using TNI to confine a single membrane protein to diffuse in an area of 300nm x 300nm. Using a GPI-anchored green fluorescence protein (GFP), which is often used a marker for cholesterol-stabilized nano-domains, to probe the membrane of PtK2 cells we detect domains that are at the same time stiffer, concentrate the protein and show slower diffusion. These align along linear feature and show convex polygons shape. These domains are further stabilized by addition of Ganglioside cross-linking to the membrane and disappear after removal of the cholesterol. Another marker, GFP-labeled transferrin receptor molecule, detects linear features and linearly demarcated areas of increased protein concentration.

Temperature-Dependent Phase Behavior of the Synaptosomal Membranes from Mammalian and Marine Invertebrate Synaptosomes
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Given reports of raft domains in rodent brain synaptosomes, we probed for their conservation in a poikilotherm, the Woods Hole sole (Solea australis). Because rafts are described as liquid-ordered phase-separated domains, we expected phase transition temperatures above body temperature if raft function is important to neuronal activity. We tested this hypothesis by comparing synaptosomes, intact nerve endings, from animals that live at two very different temperatures: mouse (body temperature-37 C) and squid (body temperature-20 C). We measured the temperature-dependence of the lipid phase of intact synaptosomes in the absence of exogenous probes by using line-width and spinning sideband intensities of lipid hydrocarbon chain resonances, using proton magic angle spinning NMR spectra as a function of temperature between 0 and 40 C. We also